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## **The molecular evolution of glioblastoma treated by gross total resection alone**

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**The molecular evolution of glioblastoma treated by gross total resection alone**

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All remaining authors declare that they have no conflict of interest.

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A better understanding of the biological evolution of gliomas and its modulation by therapeutic interventions has become a central topic <sup>1,2</sup>. However, little is known about the natural evolution of glioblastoma treated by surgery alone. Glioblastoma patients are almost always treated with radiotherapy or chemotherapy, and if not, rarely undergo second surgery.

Here, we report a series of five glioblastoma patients, who did not undergo treatment beyond surgery, but had second surgery for recurrent disease after a median of 66 days (range 36-128 days; patient 1, 128 days; patient 2, 66 days; patient 3, 95 days; patient 4, 36 days; patient 5, 38 days). Reasons included patient wish (patients 1, 2, 3) or early progression detected during radiotherapy planning (patients 4, 5). All patients had gross total resection at first surgery followed by another gross total (patients 1, 3, 4) or partial resection (<99%) (patients 2, 5) at recurrence. Median age at diagnosis was 63 years (range 50-72 years). Three patients were included in a previous study <sup>2</sup>. This study was approved by the Ethics Committee of the Canton of Zurich.

All tumors were histologically classic glioblastomas, isocitrate dehydrogenase (IDH)-wildtype <sup>3</sup>. Fig. 1A shows representative histological features of primary and recurrent tumors, focusing on vital tumor areas with microvascular proliferation. There were no differences in cellularity, predominant cell types and presence of necrosis or microvascular proliferation. Comparison of molecular profiles by gene panel sequencing <sup>4</sup> revealed few differences between primary and recurrent specimens (Fig. 1B). O<sup>6</sup>-methylguanine DNA methyltransferase (*MGMT*) promoter methylation status did not change. DNA methylation profiling revealed IDH-wildtype glioblastoma methylation class family tumors of the receptor tyrosine kinase I (RTK I), RTK II and mesenchymal (MES) subclasses <sup>5</sup>. Longitudinal methylation profiles

were available from two patients, indicating a change in DNA methylation subclass in patient 1 from RTK II at initial surgery to RTK I at recurrence, while primary and recurrent tumors of patient 4 were both assigned to RTK II (Fig. 1B). Copy number variations (CNVs) slightly differed between primary and recurrent tumors in two patients with amplification of the cyclin-dependent kinase 4 (*CDK4*) gene at recurrence in patient 1, and homozygous deletion of cyclin-dependent kinase inhibitor 2 (*CDKN2A*) found only in the initial biopsy from patient 3. Finally, differences in single nucleotide variations were seen in the tumor from patient 4, where a mutation in the epidermal growth factor receptor (*EGFR*) gene was detected in the recurrence. In addition, tumor tissue from patient 5 showed a mutation in the neurofibromatosis 1 (*NF1*) gene at diagnosis but not recurrence (Fig. 1B). Except for these two cases, molecular profiles remained stable, as also exemplified in the CNV profiles in both the initial tumor and recurrence from patient 4 (Fig. 1C), which are typical for glioblastoma, IDH-wildtype. Representative neuroimaging scans from patient 4 at diagnosis and recurrence are shown in Fig. 1D.

Thus, histopathological features, DNA copy number profiles and driver gene alterations remain remarkably stable from primary to recurrent glioblastoma in patients treated by gross total resection alone. Overall, these findings differ from longitudinal studies of glioblastomas treated with cytotoxic therapy, which frequently reveal more pronounced molecular changes at tumor recurrence, including temozolomide-induced hypermutation in a subset<sup>1,2,6-8</sup>. Thus, therapy likely modulates the molecular evolution in glioblastoma. Admittedly, the time interval between surgeries in our series was short compared with other studies<sup>1,2,6-8</sup>. Still, a better understanding of molecular changes in response to specific treatments,

including radiotherapy, alkylating chemotherapy and immunotherapy may aid clinical decision making by refining inclusion criteria for clinical trials at recurrence.

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**Fig 1. Histopathological characteristics, DNA methylation subclasses and mutational profiles, and exemplary neuroimaging features of primary (PT) and recurrent tumors (RT) of the five glioblastoma patients (1-5).** (A) Representative histological features in vital tumor areas of the five pairs of PT (left side) and RT (right side); scale bars, 50  $\mu$ m. (B) Graphic representation of the results obtained by DNA methylation profiling and next generation sequencing of a glioma-associated gene panel. Assignments to molecular subgroups as well as absence or presence of the listed aberrations are indicated in color codes as illustrated below the figure. (C) Example of copy number plots calculated from DNA methylation array data of PT and RT of patient 4; results for chromosome 1-22, X and Y are shown with the p-arm (left) and the q-arm (right) separated by dotted lines; gains/amplifications represent positive (green), losses negative (red) deviations from the baseline. (D) Axial T1-weighted gadolinium- enhanced MRI scans of patient 4 with newly diagnosed tumor (day 1) and recurrent tumor (day 34) are shown; surgery was performed at day 2 and day 38; gross total resection after first surgery was confirmed with contrast-enhanced CT.

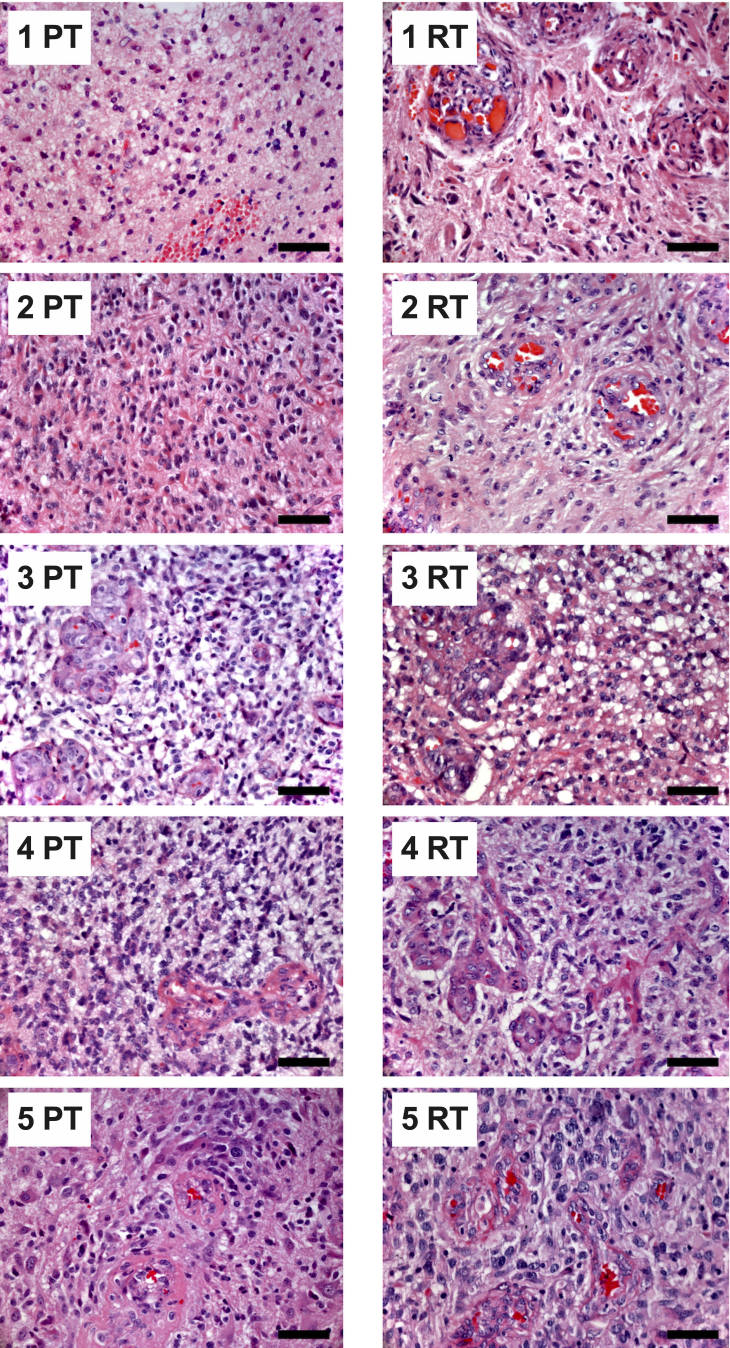
Abbreviations: amp., amplification; *CCDN2*, cyclin D2; *CDK4*, cyclin-dependent kinase 4; *CDKN2A*, cyclin-dependent kinase inhibitor 2; chr, chromosome; CNV, copy number variation; del. rear., deletion rearrangement; *EGFR*, epidermal growth factor receptor; *EGFRvIII*, variant III of EGFR; hom.del., homozygous deletion; M, methylated; MES, mesenchymal; *MGMT*, O<sup>6</sup>-methylguanine DNA methyltransferase; n.a., not available; *NF1*, neurofibromin 1; *PIK3CA*, phosphoinositide-3-kinase; *PTEN*, phosphatase and tensin homolog; *PTPN11*, protein tyrosine phosphatase non-receptor type 11; RTK, receptor tyrosine kinase; SNV, single nucleotide variation;



*TERT*, telomerase reverse transcriptase; *TP53*, tumor protein 53; UM, unmethylated; wt, wildtype.

Fig. 1

A

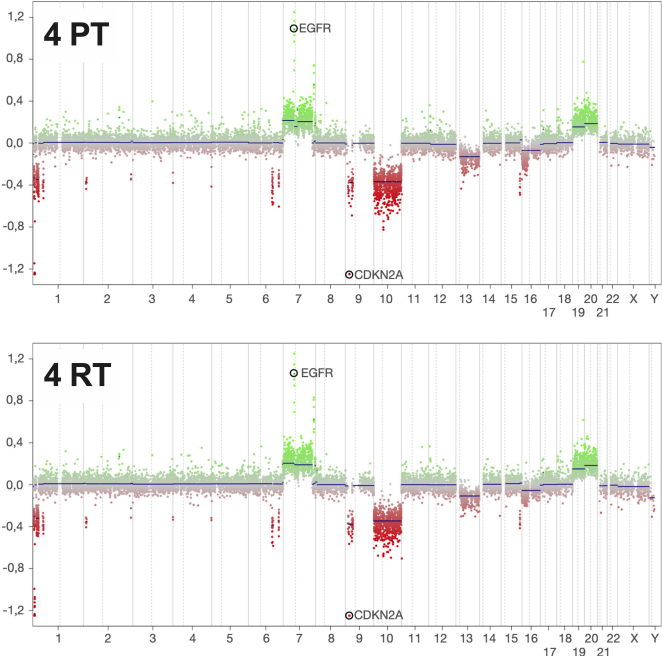


B

		1		2		3		4		5	
		PT	RT	PT	RT	PT	RT	PT	RT	PT	RT
SNV	850K										
	MGMT										
	TERT										
	TP53										
	EGFR										
	PTEN										
	PIK3CA										
	PTPN11										
	NF1										
CNV	EGFR										
	EGFRvIII										
	CCDN2										
	CDK4										
	chr7										
	chr10										
	CDKN2A										



C



D

